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Special Report

Hormonal Regulation of Protein Metabolism

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On February 5, 1989 a distinguished group of scientists, including members of A.S.P.E.N. as well as others from around the world, met at the 13th A.S.P.E.N. Clinical Congress Meeting. This meeting was convened for the purpose of discussing advances in understanding of the regulation of protein metabolism in patients, including those patients undergoing specialized nutritional support. A summary of the deliberations of that meeting, including materials presented by invited speakers, is outlined below. While it was neither the purpose of this meeting, nor of the current discussion, to exhaustively review all of the basic science and applied physiologic information relative to the topic, it is hoped that this overview will provide readers with insights into recent investigations by leading researchers.

A general characterization of the host response to injury and infection is readily evident from the many reports of hypermetabolism and hypercatabolism over the past 50 years. These hallmarks of injury, including increased resting energy expenditure and increased protein wasting exhibit some variability but appear to be, in most cases, related to the intensity of the insult. This workshop, addressing those factors that may serve to regulate protein metabolism, might be considered quite late by the perfectionist since Sir David Cuthbertson showed nearly 60 years ago that increased protein wasting accompanied long bone fracture.[1]

ALTERATIONS IN PROTEIN METABOLISM FOLLOWING INJURY

Using isotopic methodologies, Shaw has confirmed that postabsorptive normal subjects present with rates of whole body protein synthesis of around 3 g/kg/day and a corresponding rate of whole-body protein catabolism in excess of this synthesis rate which results in a net protein catabolism or protein loss of about 1.5 g/kg/day.[2,3] When stressed or catabolic patients were evaluated, there was a greater increase in whole-body protein catabolism with only a slight increase in whole-body protein synthesis. The net result of such responses was to cause an increase in net protein catabolism in highly stressed states. It is of interest to note that the severity of injury does, to some extent, correlate with measured glucose production rates and net protein catabolism. Injury severity scores up to 20 provided for a significantly increased glucose production and net protein loss. In stratifying the patients to scores up to and greater than 40, there was no further change in either glucose production or net protein catabolism, suggesting that the response to the most severe injury or other major catabolic insults is to some degree an all or none phenomenon.[3]

An evaluation of body protein metabolism in cancer patients reveals that there is no significant difference between such patients and nontumor-bearing controls during the early nonweight-losing stages of disease.[4,5] However, when patients with advanced upper gastrointestinal cancer are evaluated, there was a major increase in catabolism and a more modest increase in the synthesis reflecting responses similar to that observed in trauma (ie, an increase in net protein loss). In the severely ill patient, be it sepsis, trauma, cancer or pancreatitis, there is a net protein catabolism as manifested by an increase in whole-body protein breakdown and a lesser increase in whole-body protein synthesis. These responses in trauma and cancer patients are unlike those in normal subjects with pure uncomplicated depletion due to starvation.[6] In this instance, the patient develops a decrease in the catabolic rate and a relative conservation of protein mass.

Protein turnover rates have also been evaluated in patients during operation by obtaining a steady state infusion of isotopes immediately prior to surgery.[7] This method allowed for the determination of leucine incorporation into various tissues biopsied during surgery, as well as the calculation of whole-body protein kinetics. The fractional synthesis rate of muscle protein was found to be about 2% per day in patients without cancer and not significantly increased in patients with early disease. However, these rates were increased substantially in patients with advanced disease. The fractional synthesis rate of protein in the liver is approximately 20% per day and is also increased in patients with advanced weight-losing cancer.

The erosion of lean body mass resulting from stressed

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states is somewhat responsive to nutritional intake. The nonstressed patient may be made anabolic with total parenteral nutrition (TPN). However, the cancer patient, although considerably improved by the intervention of TPN, in many instances could not be made anabolic.[8] These findings are similar to those reported in severely stressed patients wherein large losses of protein occurred in the basal state. Although such losses could be ameliorated with the intervention of TPN, net losses of body protein continue under these conditions.[9,10] It is generally accepted that at the height of severe trauma, patients cannot be made uniformly anabolic regardless of the level of nutritional intervention. However, a more positive nitrogen balance is obtained from nutritional intervention and may be considered an asset in terms of patient care.

Since the responsiveness of the severely traumatized patient to nutritional intervention is somewhat limited, several groups have attempted to evaluate various mediators of catabolism and by employing antagonist therapy, have sought to evaluate the influence of such mediators on protein catabolism. Somatostatin, an antagonist to glucagon, naloxone, an antagonist to endorphins, ranitidine, an antagonist to histamine, and diclofenac sodium, an antagonist to prostaglandins, each caused a decrease in whole body protein catabolism and net protein loss.[11–13] Beta-adrenergic stimulation with salbutamol had no effect on net protein catabolism.[12] Similar results are observed when patients are evaluated during total parenteral nutrition.

When considering the catabolic response of trauma, both changes in protein synthesis and/or degradation must be considered. Net protein catabolism may be related to an increase in protein degradation that is offset, in part, by protein synthesis. However, there are examples where a decrease in protein synthesis may occur, such as in muscle disuse atrophy. Tischler et al.[14] has described an animal model of disuse atrophy by unweighing the hind limbs by suspension. Results obtained in this model suggest that such atrophy results from changes in cytoplasmic proteolysis with little or no role of the lysosomes. During disuse atrophy there is an increase in insulin sensitivity in the muscle due to sparing of insulin receptors. However, denervated muscle exhibits a loss of insulin receptors and a general increase in insulin resistance. Recent studies have also addressed the clinical importance of such muscle disuse in hospitalized patients[15,16] and have additionally sought to evaluate the role of submaximal exercise as a clinical adjuvant to nutritional support.

CLASSICAL HORMONES AND PROTEIN REGULATION

An exaggerated stress hormone response has been assumed to exert a major influence upon postinjury protein metabolism. In an effort to delineate the contribution of such glucose counter-regulatory hormones following injury, several research groups have evaluated the metabolic response to elevated hormone levels in normal subjects.[17,18] The metabolic effect of catecholamines, cortisol, and glucagon have been evaluated both individually as well as during simultaneous administration. These hormones mimic some components of the injury state by virtue of their effect on liver and muscle glycogen and by raising blood glucose. However, their influences on protein metabolism are not quite as clear.

The acute administration of epinephrine appears in some circumstances to be anabolic rather than catabolic.[19] The effect of epinephrine on carbohydrate and fat metabolism has been extensively discussed and it has been noted that free fatty acid levels increase in a dose response fashion to epinephrine infusion.[20–23] Since epinephrine increases glucose production one might consider epinephrine as anabolic from an energy mobilization perspective and catabolic from the perspective of increasing metabolic rate. Matthews et al.[19] has concluded that the infusion of mild to moderate doses of epinephrine produces very little effect on protein metabolism. A depression of amino acid levels is observed in concert with increased metabolic clearance for amino acids if the infusion rate of epinephrine is increased. The amino acid kinetic changes return to normal, however, once a new steady state is achieved. Hence, epinephrine does not appear to be responsible for the alterations in protein metabolism observed following injury.

Cortisol is the counter-regulatory hormone exhibiting the most potential for inducing heightened protein catabolism. It has been noted that the administration of stress-level doses of glucocorticoids results in a more negative nitrogen balance of approximately 4 g/day[17,18] and skeletal muscle appears to be a primary target for this physiological response to cortisol. Countering these observations is previous work using sham and adrenalectomized rats wherein similar urinary nitrogen excretion was observed following fracture.[24] These data imply that the acute rise in cortisol observed in stressed patients may not be critical as an absolute regulator of the protein catabolic response to injury. However, such studies do not exclude a very important permissive role of persistently high cortisol levels following injury.

In evaluating potential mechanisms involved, data concerning the interaction between insulin and cortisol upon protein metabolism suggests that these two hormones are antagonistic to each other. Manipulating insulin and cortisol levels following adrenalectomy, very little protein catabolism occurred during starvation; however, when replacing the cortisol to normal levels a marked increase in nitrogen loss was observed.[35] In the fed state, where insulin levels are high, one needs high levels of cortisol to achieve a catabolic effect. These responses were compared in normal muscle and muscle from traumatized animals. The antagonistic effects of insulin on cortisol in normal muscle resulted in a decrease in protein degradation. However, insulin failed to antagonize this response in muscle from traumatized animals. The cortisol effect on protein degradation may be expressed as even greater simply because insulin cannot antagonize it.

It is now well documented that prolonged administration of a hormone infusion combination of cortisol, catecholamines, and glucagon, does not elicit the same degree of nitrogen wasting as that observed in critically ill patients.[17,18] During a 3-day infusion of these catabolic hormones, there is a persistent hyperglycemia accompanied by hyperinsulinemia. Glucose kinetic studies suggest that an increase in the endogenous glucose production rate of a magnitude resembling that noted in stressed or traumatized patients. However, the enhancement of nitrogen excretion was modest, being on the order of about 4 g/day, and this degree of nitrogen loss is quite unlike that observed following severe trauma or injury. Comparing the responses of the hormone cocktail to cortisol alone, it has been concluded that cortisol could account for much of the nitrogen wastage in these normal subjects.[17] Similar studies reported by Bessey and colleagues[18] have demonstrated nearly identical responses to a similar infusion of counterregulatory hormones in normal subjects. Frayn[25] has also pointed out that the correlation of counter-regulatory hormonal concentrations to the peak catabolic responses are out of phase in patients. Hence, it would appear that such hormones cannot quantitatively reproduce the pronounced protein catabolism observed during the flow phase of injury.

An alteration in nitrogen balance or of urinary excretion of 3-methylhistidine is not observed in normal volunteers or diabetic patients in response to glucagon infusion. However, protein catabolism progressively increases during glucagon infusion during hypoinsulinemic conditions. Increased proteolysis and increased leucine oxidation occur under such circumstances, hence, glucagon can be considered a catabolic hormone only when insulin is deficient.[26,27]

In general, the effects of insulin on protein regulation are presumed to be a decrease in proteolysis and an enhancement of protein synthesis. However, results from studies examining *in vivo* protein metabolism do not completely support these findings. Insulin has been shown to decrease net amino acid release across the human forearm. Barrett et al[28] and Gelfand and Barrett[29] attributed this effect to a decrease in the rate of proteolysis with minimal, if any, effect on protein synthesis. Studies using isotopic techniques have examined the effect of various concentrations of insulin on whole body amino acids kinetics *in vivo* and found that insulin exerts its primary effect by suppressing proteolysis and secondarily by decreasing amino acid oxidation.[30-32]

Utilizing an *in vitro* injury model, Moss et al.[33] and Hurst et al[34] found that muscle tissue responded to insulin by an increase in protein degradation with no change in protein synthesis. In an effort to determine the effect of insulin, four different muscle types showed some inhibition of protein degradation by insulin. However, within several days, protein degradation returned to normal. Hence, this muscle insulin resistance appears to specifically be isolated to glucose metabolism and protein degradation with the data suggesting a postreceptor defect.

In an effort to clarify what may be causing such insulin resistance clinically, Tischler et al[35] have utilized plasma from normal and trauma patients and removed large molecular weight proteins prior to incubation with rat muscle. Under these circumstances, insulin had no effect on protein synthesis in tissue treated with either control or trauma plasma. However, insulin inhibition of protein degradation in the control samples was twice as great as influences on protein degraded in tissues treated with plasma from trauma patients. These data suggest the existence of other plasma factors in trauma patients that interferes directly with the influence of insulin on protein degradation.

Studies have suggested that the *in vivo* effect of insulin upon protein kinetics is dependent on the prevailing plasma amino acid level. Brown and colleagues[36] have evaluated the effect of insulin on protein metabolism, utilizing a refined high-performance liquid chromatography (HPLC) methodology to allow for the rapid measurement of amino acids and production of a euaminoacidemic state. Utilizing leucine as the plasma amino acid marker, protein kinetics were determined during continuous infusion of [1-^{14}C]leucine in human volunteers.[37] Euglycemia was also maintained with a glucose clamp procedure. Results from these overnight fasted volunteers indicates that the major influence of insulin *in vivo* was to inhibit proteolysis in a dose dependent manner. The data also indicate that there are an insulin-responsive and an insulin-unresponsive component to protein synthesis and breakdown as well as of amino acid oxidation. By utilizing this amino acid clamp technique during various levels of euglycemic hyperinsulinemia in normal subjects, these investigators have been able to demonstrate that the insulin unresponsive component was highly dependent on circulating plasma amino acids. In the presence of basal plasma amino acids (euaminoacidemia), proteolytic suppression by insulin was enhanced and hypoaminoacidemia associated with insulin treatment blunted the insulin suppression of protein breakdown.

Using a canine model, the effect of altering the prevailing glucose level has also been evaluated during a continuous infusion of insulin as well as [1-^{14}C]leucine to track protein kinetics.[36] The plasma cortisol is elevated if the animal is maintained in a hypoglycemic state. The norepinephrine, epinephrine, and glucagon are also elevated in the hypoglycemic state but not in the euglycemic group. Under such conditions plasma leucine concentrations dropped 4-fold in the euglycemic group. In this evaluation of the nonoxidative disposal of leucine in man and animals, the rate of leucine utilization, suggestive of protein synthesis was decreased and stayed down during hypoglycemia. A rebound toward basal levels occurred by the end of the study. During hypoglycemia there was a significant uptake of amino acids by the hind limb suggesting that the source of amino acids is likely from hepatic and splanchnic tissues. During euglycemic conditions, the infusion of epinephrine or norepinephrine do not reproduce these responses and combined infusions of epinephrine, norepinephrine, glucagon, and cortisone likewise fail to reproduce the above responses. It is of interest that increasing beta-endorphin levels in cerebral spinal fluid did reproduce the changes in protein kinetics (Abumrad, NN: in preparation).

Considerations in Protein Kinetic Methodologies

A possibility exists that the amino acid pool for protein synthesis, is derived from the breakdown of protein, as opposed to amino acids derived from circulating pools. In reflecting on this concern, Carraro et al[39] have utilized hippurate as a precursor for enrichment measurements to determine plasma protein synthesis rates using ^{15}N glycine, and observed that the enrichment in glycine in the liver was considerably higher than the ^{15}N enrichment in hippurate. This could mean that hippurate synthesis is not derived from a homogeneous pool of all intracellular amino acids but rather incorporating glycine derived from intracellular protein breakdown to a greater extent than that which is derived from circulating amino acids. For those measuring whole-body protein synthesis rates, this is a point of considerable concern.

In evaluating leucine kinetics in fasting, catabolic patients, the rate of leucine appearance is significantly elevated both in burn and septic patients.[40] When these studies are repeated with a hyperinsulinemic clamp, there is a significant reduction in the rate of appearance of leucine. However, when expressed as a change from the basal state, burn patients show significant increases in the rate of leucine appearance. It has been proposed that leucine appearance may be related to both an insulin-sensitive component as well as an insulin-insensitive component. Similar responses were observed regarding leucine oxidation with hyperinsulinemia and hypoglycemia. Under such circumstances, a significant increase in leucine oxidation is observed, as opposed to no effect during an euglycemia clamp. These data suggest that the reason for the sustained high rate of leucine oxidation and leucine breakdown occurring in patients during insulin clamp may relate to an impaired oxidative capacity for glucose. That this was not obviously the case is demonstrated by the increase in glucose oxidation in septic patients in a clamp setting demonstrating the same oxidation as a result of infusion of dichloroacetate, a stimulator of pyruvic dehydrogenase.[40] Dichloroacetate did not appear to have any particular effect on the rate of leucine appearance or oxidation in these patients. Hence, it can not be concluded from these data that an alteration in the postreceptor metabolisms of glucose influences such kinetic responses.

GROWTH FACTORS AND CYTOKINES INFLUENCES UPON PROTEIN METABOLISM

Growth Hormone and Somatomedins

The recent availability of recombinant human growth hormone has refocused attention upon the role of this hormone in the regulation of protein metabolism. Although it is recognized that growth hormone may, within certain tissues, exhibit growth promoting actions, it is likely that the systemic anabolic properties of growth hormone result at least in part from the induction of somatomedins.[41] Van Wyk and co-workers[41] have proposed that the actions of growth hormone fall into two broad categories: those that are synergistic with cortisol and opposite the action of insulin, and those that are insulin-like and opposed by the actions of cortisol. It is further suggested that the actions of growth hormone that are synergistic with cortisol, such as increased lipolysis and carbohydrate intolerance, may be classified as direct actions of growth hormone (GH) GH and therefore require no intermediary agent. Growth hormone directly influences some aspects of cellular physiology which are necessary for cell proliferation and protein synthesis.[42] As a consequence, it has been hypothesized that although many cells of the body may be direct targets for the growth promoting effects of GH, the basis for this action may be to induce conditions which render such cells susceptible to other growth factors.[43]

By contrast to those metabolic events not requiring an intermediary peptide, there is increasing evidence to suggest that several growth promoting effects of growth hormone, including cell proliferation and protein synthesis in various tissues, are directly mediated by the somatomedins. This category of hormonal peptide growth factors is likely composed of two proteins, insulin-like growth factors I (IGF-1, Somatomedin C) and II. IGF-1 is more growth hormone dependent and less insulin-like than is IGF-II. The increased transcription of mRNA for IGF-1 is very dependent upon the presence of growth hormone in most somatic tissues such as liver and muscle.[44]

Growth Hormone

Recent clinical studies have clearly established that the administration of recombinant growth hormone can establish a state of nitrogen retention under conditions of hypocaloric[45] as well as complete[46–48] intravenous nutritional support. Additionally, the optimal promotion of systemic protein accrual in response to growth hormone appears to exhibit a relationship to the adequacy of substrate provision.[49]

Although recent studies demonstrate that the administration of growth hormone can improve nitrogen retention and enhance whole body protein synthesis during some catabolic conditions,[50] such observations may not be uniformly attained during more severe stress states or in critically ill patients. A number of theories as to this resistance to anabolism have been proposed. Among such factors, the existence of inhibitory factors, such as that observed during steroid administration,[51] may serve to limit the biological activity of growth hormone in catabolic and injured patients.

Somatomedins

It presently appears that many of the protein anabolic properties attributable to growth hormone are mediated via the induction of somatomedins, with somatomedin-C (IGF-1 being the principal mediator of structural protein regulation in adults. The measurement of IGF-1 has also been proposed as a sensitive marker for nutritional status,[52,53] with circulating levels being quite sensitive to the adequacy of both non-protein and essential amino acid provision.[54–56] It is also increasingly evident that the regulation of tissue metabolic function by IGF-1 is dependent upon a complex interaction with IGF-1 binding proteins that serve to transport the protein and present it to the cell membrane.

Clinical trials utilizing IGF-1 as an adjuvant therapy to nutritional support are underway. Early experimental studies in catabolic animal models suggest that this protein, when continuously administered, is very effective in promoting lean tissue protein accrual (Ng E-H, et al: Insulin-like growth factor-1 decreases tumor aneuploid fraction and preserves host lean tissue mass in a rat sarcoma model. Surg. Forum, in press). However, it remains speculative as to whether these growth mediators will prove effective in promoting tissue protein accrual in either unstressed or stressed clinical conditions.

CYTOKINES

In addition to the contribution of the classical neurohumoral endocrine system, there is considerable evidence to suggest that endogenous products of the immune system are responsible for many somatic tissue changes resulting from injury or inflammation. Such mediators differ from classical hormones in that their production and activity are exerted predominantly in the local tissue environment rather than by influencing cellular function by circulating to distant tissue sites. The cytokine mediators are pluripotent in activity, with the ability to induce a number of systemic and tissue specific responses depending upon the model chosen for study and the range of doses examined.[58–60] It is also increasingly evident that many of these mediators are produced in multimeric form and that secreted, as well as cell associated, species of such proteins can elicit biological responses.[61,62] Observations that systemic hemodynamic responses as well as other metabolic parameters reminiscent of clinical injury[63] attend cytokine administration have served to direct investigation into their role as important mediators of postinjury protein metabolism. Although it can be reasonably assumed that such mediators act in concert with classical neuroendocrine hormones in the regulation of tissue protein metabolism, this area of investigation is preliminary in nature. Additionally, it is increasingly evident that the cytokine mediators interact with each other in complex ways. As a consequence, it is difficult to ascribe a single activity to a given cytokine *in vivo*. At present, information referable to the "presumed" influence of cytokine activity must be considered in light of the overlap of function these mediators likely exhibit *in vivo*.

Interleukin-1

The presumed role for IL-1 as a principle mediator of somatic tissue protein metabolism arose from early studies of partially purified protein wherein it was observed that monocyte preparations of increased *in vitro* muscle amino acid release and prostaglandin E_2 production.[64] *In vivo*, the administration of this partially purified monocyte preparation were capable of inducing amino acid oxidation and enhanced myofibrillar protein breakdown.[65] Although this influence was also observed in plasma from febrile patients and initially attributed to a low molecular weight species of IL-1,[66] subsequent studies have failed to observe this *in vitro* alteration of *in vitro* muscle protein regulation using recombinantly derived forms of IL-1.[67] Recent studies evaluating the influence of IL-1 administration upon myofibrillar gene transcription have likewise failed to demonstrate a specific influence of this cytokine.[68] Interestingly, this latter study demonstrated that IL-1 administration induced systemic alterations in protein metabolism similar to those induced by endotoxin. As a consequence, it is likely that IL-1 is of importance in the systemic protein response to infection although induction of other mediators or synergy with other hormones or cytokines is probably necessary.

The ability to demonstrate circulating IL-1 activity has proven problematic as recent studies have failed to consistently detect elevated levels of this mediator following endotoxin administration to man.[69] The circulating form of this cytokine is not consistently observed during clinical circumstances where other demonstrable abnormalities of substrate metabolism would be observed,[70,71] suggesting that circulating IL-1 does not appear to be a common clinical phenomenon. Rather, the documentation that this cytokine also exists in a cell-associated form emphasizes the requirement for studies directed at local tissue production of the cytokine.

Tumor Necrosis Factor

As a protein which appears to exhibit direct influences upon tissue lipoprotein lipase as well as upon glycolytic pathways, it has been proposed that tumor necrosis factor (TNF) would also exert a direct effect on protein metabolism.[60] The exogenous administration of TNF does induce both anorexia and a redistribution of protein from peripheral tissue stores to liver.[68] Although acute changes in peripheral tissue efflux of amino acids are observed following bolus injection of TNF to animals[72] and man,[73] such studies have been unable to dissect a direct influence of TNF distinct from that exerted by other cytokines[74] or by counter-regulatory hormones such as cortisol.[73] Although it has been suggested that exogenous TNF does exert a direct influence upon the breakdown of skeletal muscle *in vivo*,[75] such effects are not observed *in vitro*.[67]

The ability to detect circulating TNF under clinical conditions has also proven elusive.[76] It is likely that the cell-associated forms of the protein are of greater importance in the regulation of regional protein homeostasis. A recent study has demonstrated that capacity of reticuloendothelial tissues to produce this cytokine under appropriate clinical conditions.[77] It is also evident that active forms of the protein are being continuously produced in such tissues[78] during normal as well as stressed conditions. Hence, it remains to be determined whether TNF will exhibit the capacity for directly influencing structural protein metabolism under normal as well as stressed circumstances.

Interleukin-6

The most recently identified cytokine which appears to represent a significant regulator of acute phase protein synthesis is Interleukin-6 (IL-6) which appears to share both independent, as well as overlapping, activity with IL-1 and TNF.[58] Like these other cytokines, IL-6 appears to be an endogenous pyrogen.[79] Circulating levels of this cytokine appear to more closely correlate with the degree of injury than does IL-1 or TNF.[80] Such correlations appear to occur during a wide variety of catabolic conditions, including malignancy,[81] infection,[82] and severe injury.[80]

The most clearly defined role for IL-6 is that of a proximal signal for the initiation of hepatic acute-phase protein synthesis. This ubiquitous role of IL-6 seems to cross several categories of acute phase proteins and likely interacts with other cytokines in this regard. It is increasingly evident that several cytokines can exert different influences upon hepatic protein synthesis, with differential responses being observed in the presence of other local mediators. While it is tempting at present to ascribe a possible role of IL-6 in the regulation of other protein pathways, there is currently no evidence to suggest that this cytokine represents the elusive macrophage protein which increases myofibrillar protein degradation.

Hormone and Cytokine Interactions

Recent evidence suggests that the cytokine class of mediators may influence the release of other hormonal agents important in the regulation of protein metabolism. While the exogenous administration of TNF and IL-1 are clearly associated with the release of counterregulatory hormones such as epinephrine and cortisol,[63,72,74,83] the ability of these mediators to influence the production or release of these hormones at very low concentrations remains undefined. One intriguing aspect of cytokines would appear to be their ability to exert regulatory influences upon hypothalamic[84] or pituitary[85] secretion of regulatory peptides. Hence, it would certainly appear that locally produced cytokines such as TNF and IL-1 may exert an indirect influence upon systemic protein metabolism via alteration of classical hormone responses.

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